

- 53 -

We claim:

1. A method of treating an ACE2 decreased state, comprising administering to a mammal having that condition a therapeutically effective amount of an ACE2 agonist.
- 5 2. A method according to claim 1 in which the mammal is a human.
3. A method of any one of claims 1 or 2, wherein the decreased ACE2 state is associated with a disorder selected from a group consisting of hypertension, congestive heart failure, chronic heart failure, acute heart failure, myocardial infarction, arteriosclerosis and renal failure and lung disease.
- 10 4. A method for gene therapy for an ACE2-decreased state, comprising delivering an effective amount of a transgene coding to an organ.
5. A method according to claim 4, wherein the affected organ is the heart or kidney or lung or blood vessels.
- 15 6. The method of any one of claims 4 or 5, wherein the ACE2 transgene is administered to the patient in a gene therapy vector.
7. The method of claim 6, wherein the gene therapy vector comprises a viral vector.
8. The method of any one of claims 4-7, wherein the patient is a human.
- 20 9. The method of any one of claims 4-8, wherein the ACE2-decreased state is associated with a disorder selected from a group consisting of hypertension, congestive heart failure, chronic heart failure, acute heart failure, myocardial infarction, arteriosclerosis and renal failure and lung disease.
- 25 10. A non-human mammal comprising the gene ACE2 wherein one allele of the gene has been disrupted.

- 54 -

11. A non-human mammal comprising the gene encoding ACE2 wherein both alleles of the gene have been disrupted.
12. A non-human mammal comprising a disrupted ACE2 mutation, wherein the disruption results in a null mutation of the gene encoding ACE2.
- 5 13. The non-human mammal of any of claims 10 to 12 which is a rodent.
14. The non-human mammal of claim 13 which is a mouse.
15. The non-human mammal of claims 10 to 14 characterized by hypertension or cardiac contractility defect or kidney defects or increased sensitivity to lung injury.
- 10 16. A nucleic acid comprising an ACE2 knockout construct.
17. A vector comprising the nucleic acid of claim 16.
18. A murine embryonic stem cell line comprising the nucleic acid of claim 17.
19. A method of screening compounds that modulate hypertension and 15 cardiac contractility and kidney failure and lung injury comprising introducing the compounds into the non-human mammal of any of claims 11 to 14 and determining the increase or decrease in blood pressure and/or cardiac contractility and or kidney functions and/or lung infection.
20. A method for screening a compound that is an agonist of ACE2 activity, comprising:
 - a. providing: i) a purified preparation comprising ACE2, ii) a substrate, and iii) a test compound;
 - b. mixing said ACE2 and said substrate under conditions such that said ACE2 can act on said substrate to produce a product, wherein said mixing is 25 done in the presence and absence of said test compound; and

- 55 -

- c. measuring directly or indirectly the amount of said product produced in the presence or absence of said test compound.
- 21. The method of claim 20, wherein said substrate is angiotensin I or angiotensin II.
- 5 22. The method of claim 20 wherein said product comprises Ang1-9 or Ang1-7.
- 23. A compound isolated according to claims 20 to 22.
- 24. The use of an ACE2 activator as a pharmaceutical substance.
- 25. The use of an ACE2 activator for treatment of cardiovascular disease
10 or kidney disease or lung disease.
- 26. The use of an ACE2 activator for preparation of a medicament for treatment of cardiovascular disease or kidney disease or lung disease.
- 15 27. A method of medical treatment of cardiovascular disease or kidney disease or lung disease in a mammal, comprising administering to the mammal in need of treatment an effective amount of an ACE2 activator.
- 28. The method of claim 27, wherein the ACE2 activator is co-administered with an ACE inhibitor.
- 29. An isolated nucleic acid molecule encoding an ACE2 polypeptide, the nucleic acid comprising a nucleotide polymorphism upstream or downstream
20 of the ACE2 nucleic acid coding region, wherein the polymorphism reduces ACE2 expression compared to wild type ACE2.
- 30. An isolated nucleic acid molecule according to claim 1 wherein the polymorphism is selected from the group consisting of at least one of ACE2a-ACE2m.

- 56 -

31. A method of detecting an ACE2 decreased state in an animal comprising obtaining a DNA sample from the animal and identifying a nucleic acid of claim 29 or 30 in the DNA sample.
32. A method for diagnosing a disease or a predisposition to a disease
5 characterized by an ACE2-decreased state in an animal comprising identifying a nucleic acid of claim 29 or 30 in a DNA sample from the animal.
33. A method according to claim 31 or 32 comprising determining whether the animal is homozygous or heterozygous for the nucleotide polymorphism.
34. A method of any one of claims 31 to 33, wherein the ACE2 decreased
10 state is associated with a cardiovascular disease or a kidney disease or lung disease selected from a group consisting of hypertension, congestive heart failure, chronic heart failure, acute heart failure, myocardial infarction, arteriosclerosis and renal failure.
35. A polynucleotide comprising a sequence which binds specifically to (i)
15 region upstream or downstream of an ACE2 nucleic coding region wherein region is proximate to a nucleotide polymorphism that decreases ACE2 expression
36. The polynucleotide of claim 35, comprising 8 to 10, 8 to 15, 8 to 20, 8 to 25, 25 to 50, 50 to 75, 50 to 100, 100 to 200, 200 to 500 or 500 to 1000 nucleotide
37. The polynucleotide of claim 35 or 36, wherein the nucleic acid specific
20 binds proximate to one of ACE2a-ACE2m under high stringency hybridization conditions.
38. The polynucleotide of claim 37, wherein the stringent hybridization conditions comprise 0.1XSSC, 0.1% SDS at 65°C.
39. The polynucleotide of claim 35, comprising a sequence complementary
25 to an ACE2 polymorphism.
40. The polynucleotide of claim 39, comprising a sequence selected from the group consisting of:

- 57 -

- a. 8-50 nucleotides of an upstream or downstream region of ACE2 which is proximate to a nucleotide polymorphism, wherein the sequence includes one of ACE2a-ACE2m and comprises all or part of one of the sequences in Figure 11.
- 5 b. a sequence that is complementary to a sequence specified in (a); and
- c. a sequence having at least 70% sequence identity to a sequence in (a) or (b), wherein the sequence having identity is capable of hybridization to ACE2 under high stringency hybridization conditions.
- 10 41. The polynucleotide of claim 35, wherein the nucleic acid is capable of use as a probe in a hybridization assay.
42. The polynucleotide of claim 41, wherein the nucleic acid sequence is detectably labeled.
43. The polynucleotide of claim 42, wherein the detectable label comprises:
 - 15 a. a fluorogenic dye; and/or
 - b. a biotinylation modification; and/or
 - c. a radiolabel.
- 20 44. An ACE2 genotyping kit comprising a detection agent for detecting the presence of an ACE2 polymorphism in a nucleic acid sample derived from an animal.
45. The kit of claim 44, wherein the detection agent comprises a nucleic acid and/or a restriction enzyme.
46. The kit of claim 44, further comprising a biological sample container for holding the detection agent.

- 58 -

47. The kit of claim 44, further comprising a plate having a plurality of wells and having bound thereto probes having a nucleic acid sequence which specifically binds to an ACE2 sequence including an ACE2 polymorphism.
48. The kit of claim 44, further comprising an amplification agent for amplifying the nucleic acid.
49. The kit of claim 48, wherein the amplification agent amplifies a region of ACE2 nucleic acid proximate to an ACE2 single nucleotide polymorphism selected from the group of ACE2a-ACE2m.
50. The kit of claim 48, wherein the amplification agent comprises a primer set, wherein each primer is a nucleic acid that will specifically bind proximate to, and/or cause elongation through, one of ACE2a-ACE2m.
51. The kit of claim 144, for detecting that the animal has or is at risk of an ACE2 decreased state disease.
52. The kit of claim 51, wherein the disease comprises cardiovascular disease or kidney disease or lung disease or affects blood vessels.
53. The kit of claim 52, wherein the disease is selected from the group consisting of hypertension, congestive and dilative chronic heart failure, acute heart failure, myocardial infarction, coronary artery disease, arteriosclerosis, and renal failure and lung disease.
54. A method of ACE2 genotyping an animal comprising:
 - a. obtaining an ACE2 nucleic acid sample derived from the animal including regions upstream and downstream of the ACE2 coding region; and
 - b. detecting a region of an ACE2 nucleic acid that includes an ACE2 single nucleotide polymorphism.
55. The method of claim 54, wherein the nucleotide polymorphism is selected from the group consisting of ACE2a-ACE2m.

- 59 -

56. The method of claim 55, comprising determining whether the animal is homozygous or heterozygous for the ACE2 polymorphism.
57. The method of claim 56, wherein the animal is a human and the ACE2 genotype is used to determine if that the animal has, or is at risk of an ACE2 decreased state disease.
58. The method of claim 57, wherein the disease comprises a cardiovascular disease or kidney disease or lung disease or effects blood vessels.
- 10 59. The method of claim 54, wherein the nucleic acid is obtained by amplifying the nucleic acid from the animal.
60. The method of claim 59, wherein the nucleic acid is obtained by amplification with all or part of the polynucleotide of any of claims 7 to 15.
61. The method of claim 54, wherein the detection step comprises determining the nucleotide sequence of the ACE2 nucleic acid.
- 15 62. The method of claim 54, wherein the detection step comprises contacting the nucleic acid with the polynucleotide of any of claims 7 to 15 under high stringency conditions.
63. The method of claim 62, wherein the polynucleotide will selectively hybridize proximate to (i) a region of ACE2 nucleic acid that includes a single 20 polymorphism distinctive of an ACE2 polymorphism.
64. The method of claim 54, wherein the detecting step comprises:
 - a. performing a restriction endonuclease digestion of the nucleic acid, thereby providing a nucleic acid digest; and
 - b. contacting the digest with the polynucleotide.

- 60 -

65. The method of claim 64, wherein the hybridization occurs either during or subsequent to PCR amplification and the analysis is by "Real-Time" PCR analysis, or fluorimetric analysis.
66. The method of claim 64, wherein the detecting step includes size
5 analysis of the nucleic acid.